BOOK REVIEW

Cell-free protein synthesis: Methods and protocols, edited by Alexander S. Spirin and James R. Swartz. 2008. Wiley-VCH, Weinheim, Germany. 262 pp. $110 (hardcover)

Anyone who has ever spent time in a biochemistry research laboratory can tell you the most problematic reagent in protein science is the protein. Cloning, expression, inclusion bodies, purification, and solubility are just a few of the many pitfalls awaiting the bench scientist on the journey from brilliant idea to published manuscript. Indeed, the value of purified and stable protein became clear (and monetarily quantified) to me during a heavy metal co-crystallization experiment. A back-of-the-envelope-calculation revealed that my protein was worth four orders of magnitude more than the gold I was worried about wasting! Needless to say, obtaining the necessary quantities of protein is often the rate-limiting step in biochemical experimentation. Thankfully, there are many new expression technologies being developed to overcome this barrier.

Perhaps most intriguing are cell-free techniques (or in vitro expression), in which the cell is dispensed with altogether, and the protein expression reaction is carried out using reconstituted translation machinery free of the constraints of cellular viability. *Cell-free protein synthesis: Methods and protocols* is a series of contributions that give an overview of the field including background and history, an in-depth look at the current protocols, and examples of recent successful applications. The book is edited by Alexander Spirin and James Swartz, two of the foremost leaders in the field. Chapter 1, written by the editors, is an introduction. The cell-free reaction consists of a diverse array of biological and chemical components and this chapter is a must-read for anyone new to this science. It describes the evolution of the physical construction of the reaction, the major organismal ''platforms'' for preparing extracts, and the basis of inclusion for other chemical constituents, including techniques for extending the reaction lifetime using a nucleotide regeneration scheme. Following the introduction, the book is roughly divided in two parts: cell-free reaction development/optimization and the application of cell-free techniques.

Chapter 2 is an interesting look at the bottom-up approach to cell-free biology, that is, a protein translation process in which all reaction components (ribosomes, elongation factors, initiation factors, etc.) are purified independently and then reconstituted. This system is an explicitly defined reaction mixture and offers the unique opportunity to study translation (particularly chaperone function) free of the cellular milieu. Chapter 3 describes a system for expression from PCR templates. Such a system could be adapted, in a high-throughput manner, to the functional characterization of entire proteomes. Those looking to bring cell-free techniques into their own laboratory setting may want to pay particular attention to Chapters 5 and 7. These chapters, which focus on bacterial and eukaryotic extracts, respectively, offer up the most practical information in the volume. This includes extract preparation, reaction setup, dialysis, and robotic automation. Much of this practical know-how is not available in the published literature.

The second half of the book focuses more on applications. One strength of cell-free expression is the ability to easily incorporate labeled amino acids, and not surprisingly many of the successes have come in structural biology. Perhaps most impressive is the workflow presented by Kigawa et al. in Chapter 6. Here, a cell-free based high-throughput pipeline is outlined that has led to the NMR determination of nearly 1300 structures. Another achievement has been the expression of integral membrane proteins. Integral membrane protein characterization has significantly lagged behind soluble proteins, and Chapters 8 and 9 show that cell-free techniques can be used for the expression of these challenging targets, including even G-protein-coupled receptors. A discussion of the effect of detergents and lipids on the reaction mixture and subsequent protein functionality is also useful. Chapters 10 and 11 focus on using cell-free expression to study ''clonal'' populations of proteins. In such a setup, single DNA messages can be amplified, transcribed, and translated independently in a microplate or gel and the resulting protein assayed. This holds particular promise in the field of protein evolution and engineering. A genetic library of high-sequence diversity can be used to express many isoforms of a protein, which can in turn be assayed, and the desirable sequences recovered. Finally, the book closes with a look

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at engineering large-scale (1L+) reactions for industrial production, particularly of therapeutics.

Nearly 50 years ago, in its infancy, cell-free expression was harnessed by Nirenberg and colleagues to unravel the genetic code. Spirin and Swartz have assembled here the evolution of this powerful technique, its state-of-the-art, and future applications. The text is clear, readable, and of sufficient detail that protocols can easily be executed by the reader. *Cell-free protein synthesis* is wholeheartedly recommended for anyone in the field or interested in learning more about protein expression.

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